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Clinical Significance of TT Virus Infection in Patients With Chronic Liver Disease and Volunteer Blood Donors in Egypt

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Clinical significance of TT virus (TTV) infection was investigated in Egyptian patients with chronic liver disease and volunteer blood donors by a cross sectional analysis. TTV DNA in serum was assessed by a semi-nested polymerase chain reaction. The prevalence of TTV DNA did not differ among patients with chronic hepatitis B (11/24, 46%), chronic hepatitis C (22/72, 31%), or schistosomal liver disease (14/39, 36%). No difference in prevalence was found between blood donors (32/109, 29%) and each of the patient groups. Clinical background including mean age, sex distribution, history of blood transfusion, and mean level of alanine aminotransferase did not differ between TTV DNA-positive and -negative individuals in any of the study groups. Ultrasonographic evidence of liver cirrhosis was similar between TTV-positive and -negative patients in each of the chronic liver disease groups. TTV infection was not associated with hepatitis B or C virus infection in blood donors. The only significant difference observed was the lower concentration of serum HCV RNA in TTV DNA positive compared with negative patients with chronic hepatitis C (3.0 ± 1.4 vs. 4.0 ± 0.9 log copies/ml, $P < .001$). In conclusion, TTV infection was not associated with either past history of blood exposure or infection with bloodborne hepatitis viruses in Egypt. No clinical significance of TTV was found in the present study. However, a reciprocal interaction was suggested between TTV and HCV replication. ***J. Med. Virol. 60:177–181, 2000.***

KEY WORDS: chronic hepatitis; hepatitis C virus; hepatitis B virus

INTRODUCTION

Considerable research has been carried out to find an agent (or agents), other than hepatitis viruses A to E, which is involved in the pathogenesis of liver disease of unknown origin. These liver diseases include non-A to E post-transfusion hepatitis [Krawczynski, 1997], cryptogenic hepatitis [Kodali et al., 1994], and non-A to E fulminant hepatic failure [Feray et al., 1993]. One consequence of such studies was the isolation of TT virus (TTV) [Nishizawa et al., 1997], which was found to be associated with elevated transaminases in three of five patients with post-transfusion hepatitis of unknown etiology. It has been suggested that TTV is a nonenveloped parvovirus [Okamoto et al., 1998b] or a circovirus [Takahashi et al., 1998b; Mushahwar et al., 1999] with a single-stranded DNA that has two possible opening reading frames capable of encoding 770 and 202 amino acids, respectively [Okamoto et al., 1998b].

TTV infection has been reported from different areas of the world, including Asia [Okamoto et al., 1998b; Tanaka et al., 1998; Orii et al., 1999], Europe [Höhne et al., 1998; Naoumov et al., 1998; Simmonds et al., 1998], Middle and South Africa [Prescott and Simmonds, 1998], North America [Charlton et al., 1998], and South America [Niel et al., 1999]. Furthermore, TTV has been detected in a variety of liver diseases, including non-A to E hepatitis [Nishizawa et al., 1997; Poovorawan et al., 1998; Orii et al., 1999], fulminant hepatitis [Charlton et al., 1998; Poovorawan et al., 1998], and hepatocellular carcinoma [Poovorawan et al., 1998; Yamamoto et al., 1998].

In the present study, the clinical significance of TTV infection was investigated in patients with chronic liver disease in Egypt, a North African country with a rather high prevalence of hepatitis C virus (HCV) infection [El Gohary et al., 1995; Arthur et al., 1997] as

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well as of hepatitis B virus (HBV) infection and schistosomiasis [Kamel et al., 1994].

PATIENTS AND METHODS

Study Groups

All patients involved in the present study were seen at the Suez Canal University and Suez Canal Authority Hospitals, located in Ismailia, Egypt, between February 21, and March 24, 1998.

The first group consisted of 96 patients with chronic hepatitis seen in the liver disease clinics during the observation period. Of these, 24 were diagnosed with chronic hepatitis B, 72 with chronic hepatitis C. There were 47 men and 49 women with a mean age of 46.0 years (range = 27–70 years).

The second group consisted of 39 patients with schistosomal liver disease seen in the liver disease clinics during the observation period. There were 28 men and 11 women with a mean age of 43.8 years (range = 22–65 years). Eighteen of the 39 patients had concomitant HCV infection (positive for HCV antibody and HCV RNA), and 8 had evidence of HBV infection (positive for hepatitis B surface antigen [HBsAg]).

The third group consisted of 109 volunteer blood donors who were selected randomly from 319 volunteer blood donors at the blood bank of the Suez Canal University Hospital during the observation period. There were 98 men and 11 women with a mean age of 29.4 years (range = 18–51 years). The predominance of men among blood donors is common in Egypt due to various cultural factors.

A standard interview was used to investigate social and clinical backgrounds in all of the study groups. Ultrasonographic assessment of the abdominal region was conducted in all patients with chronic liver disease for evidence of liver cirrhosis and schistosomal liver disease.

Informed consent was obtained from all of the patients involved in the study.

Working Definitions

Chronic hepatitis C was diagnosed on the basis of positive HCV antibody and HCV RNA in the presence of elevated level of alanine aminotransferase (ALT) for 6 months or longer. Supporting data were obtained from abdominal sonographic evidence of chronic changes in the liver. Chronic hepatitis B was diagnosed on the basis of the presence of HBsAg and elevated ALT for 6 months or more. Supporting data were obtained from abdominal sonographic evidence. Schistosomal liver disease was diagnosed on a past history of schistosomal infection (stools or urine positive for schistosomiasis) in the presence of ultrasonographic evidence of periportal fibrosis (grade II to III portal thickening) [Nooman et al., 1995].

Liver cirrhosis was diagnosed on clinical evidence of chronic hepatic insufficiency or ultrasonographic features of liver cirrhosis. The ultrasonographic evidence included hyperechogenicity of hepatic texture compared to kidney cortex with diffuse parenchymal changes, hepatic atrophy with attenuated vasculature, irregular contour of the liver denoting nodular regeneration, and portal hypertension with splenomegaly [Kawamura et al., 1992].

Laboratory Tests

The serum ALT was measured using an autoanalyzer, with the normal range set between 7 and 45 IU/L. Second generation HCV antibody, HBsAg, anti-HBs, hepatitis B e antigen (HBeAg), and HB core (HBc) antibody were measured using commercially available enzyme-linked assay kits (International Reagents, Kobe, Japan).

HCV RNA in Serum

HCV RNA was detected by nested reverse transcription-polymerase chain reaction (RT-PCR) using two sets of primers synthesized from the 5′ noncoding region of the HCV genome as reported previously [Matsumoto et al., 1994].

The concentration of HCV RNA was measured by a competitive nested RT-PCR using methods reported previously [Matsumoto et al., 1994].

TTV DNA in Serum

TTV DNA was detected by a semi-nested PCR using the primer sets as reported previously [Okamoto et al., 1998a]. The total nucleic acids were extracted from 100 μl of serum and resolved in 20 μl of Tris-EDTA buffer. After heating at 95°C for 15 min, the solution was chilled on ice. A half portion of the extract was subjected to semi-nested PCR. The first round PCR was carried out in 35 cycles (96°C, 30 sec; 60°C, 45 sec; 72°C, 45 sec; with an additional 7 min in the last cycle) with the primers NG059 and NG063. The second round PCR was carried out in 25 cycles with the primers NG061 and NG063. In each PCR assay one negative and two positive controls were tested together with the serum samples.

Statistical Analysis

Statistical analyses were conducted using Student's t-test, chi-square test, and Fisher's exact test. The level of statistical significance was set at $P = .05$.

RESULTS

Prevalence of TTV DNA in Different Study Groups

The results showed a generally high prevalence of TTV DNA in all groups studied (Table I). No difference in the prevalence of TTV DNA was found among groups, including that of apparently healthy volunteer blood donors. Among patients with schistosomal liver disease, the prevalence of TTV DNA was about two times higher in those with concomitant HCV infection than in those with schistosomal liver disease alone, but the difference was not statistically significant ($P = .083$).

TABLE I. Prevalence of TTV DNA in Different Study Groups

Groups	Total no.	TTV DNA positive no. (%)	P*
Chronic hepatitis	96	33 (34.4)	>0.2
Type B	24	11 (45.8)	0.118
Type C	72	22 (30.5)	>0.2
Schistosomal liver disease	39	14 (35.9)	>0.2
With HCV infection	18	9 (50.0)	0.083
Without HCV infection	21	5 (23.8)	>0.2
Volunteer blood donors	109	32 (29.4)	

*P values were calculated by chi-square test against the data of volunteer blood donors.

Clinical Significance of TTV Infection

Clinical and virological backgrounds were compared according to the status of TTV infection in patients with chronic hepatitis B and C (Table II), in patients with schistosomal liver disease (Table III), and in blood donors (Table IV). The mean age, sex, distribution, and history of blood transfusion did not differ between TTV DNA-positive and -negative individuals in any of the groups studied. The severity of liver disease in terms of ALT level and ultrasonographic evidence of cirrhosis did not differ between TTV DNA-positive and -negative patients with chronic hepatitis B, chronic hepatitis C, or schistosomal liver disease. The positive rate of serum HBeAg, which is related to the activity of HBV replication [Hoofnagle et al., 1981], was comparable between TTV DNA-positive and -negative patients with chronic hepatitis B. In contrast, the serum concentration of HCV RNA was significantly lower in patients with TTV DNA with associated chronic hepatitis C than in patients without TTV. Positive rate of HBV and HCV related serum markers were comparable between TTV DNA-positive and -negative blood donors.

DISCUSSION

Previous clinical studies of TTV infection have been made using PCR for detection of serum TTV DNA. A primer set for the PCR was first reported by Nishizawa et al. [1997], after which new primer sets were reported by Okamoto et al. [1998b] and Takahashi et al. [1998a]. In the present study, the primer set reported by Okamoto et al. [1998b] was used, because most studies of TTV infection worldwide have used this primer set.

Several studies, including the present study, have shown that Egypt is highly endemic for HCV infection [El Gohary et al., 1995; Arthur et al., 1997], and HBV and schistosomal infections [Kamel et al., 1994]. Similarly, TTV infection was found to be highly prevalent (29%) in Egyptian blood donors. However, TTV was found not to be associated with either previous injection for treatment of schistosomiasis or infection with known bloodborne hepatitis viruses. These results suggest that TTV infection has been transmitted in the Egyptian population in a manner different from that for HCV, HBV, and schistosomal infection.

The finding that the prevalence of TTV DNA in patients with chronic hepatitis was comparable to that in blood donors in Egypt was different from that of Orii et al. [1999], who found that the prevalence of TTV DNA was significantly higher in patients with chronic hepatitis than in blood donors in Japan. Such a difference can be attributed partly to the relatively higher prevalence of TTV DNA in Egyptian blood donors, and partly to the higher prevalence of TTV DNA in Japanese patients with chronic hepatitis C (60%). However, our results are in agreement with those of Naoumov et al. [1998], who found no difference in TTV prevalence between patients with chronic liver disease and blood donors in the United Kingdom. This difference in results between Japan and the United Kingdom cannot be attributed to a genotypic difference, because the DNA sequence was found to be similar in both countries [Naoumov et al., 1998].

Clinical background including mean age, sex, and transfusion history did not differ between TTV DNA-positive and -negative patients with chronic liver diseases; a similar tendency was observed by Orii et al. [1999]. Furthermore, no association was found between the presence of TTV infection and the severity of liver diseases in terms of serum ALT level or ultrasonographic evidence of cirrhosis. These findings agree with the data reported by other investigators [Naoumov et al., 1998; Takahashi et al., 1998a; Orii et al., 1999; Umemura et al., 1999]. Although further studies are required, our results did not indicate any clinical significance of TTV.

It is noteworthy that the concentration of HCV RNA was significantly lower in patients with HCV and TTV infections than in those with HCV infection alone. A reciprocal relationship between TTV and HCV infections was reported in a study conducted in an area highly endemic for hepatitis C [Umemura et al., 1999], where TTV infection was less prevalent in individuals with HCV infection than in those without this infection. TTV is thought to replicate in the liver [Okamoto et al., 1998b]. Thus, it is possible that a reciprocal relationship exists between TTV and HCV replication, similar to that reported between HBV and HCV [Pontisso et al., 1993]. Further studies will be needed to clarify this issue.

Patients with schistosomal liver disease in Egypt are at risk for HCV infection due to the fact that in the past such patients had been treated with antischistosomal injections using shared syringes, which is considered to be an important risk factor responsible for the transmission of hepatitis viruses in Egypt [Hyams et al., 1987; Darwish et al., 1993]. Indeed, in our study, as many as 46% of the schistosomal patients were found to be infected concomitantly with HCV and this rate was significantly higher than in blood donors. On the other hand, the rate of TTV infection in patients with schistosomal liver disease was found to be comparable to that of blood donors. Furthermore, TTV infection was not associated with transfusion history in the schistosomal group, and was not associated with HCV infection in the blood donors. These findings raise the

TABLE II. Comparison of Clinical and Virological Backgrounds Between TTV DNA Positive and Negative Patients With Chronic Hepatitis*

Background	TTV DNA positive	TTV DNA negative	*P*
Chronic hepatitis B	$n = 11$	$n = 13$	
Mean age (years)[a]	46 ± 13	42 ± 9	>0.2[c]
Sex (male:female)	8:3	10:3	>0.2[d]
History of blood transfusion[b]	8 (73)	10 (77)	>0.2[d]
Mean ALT level (IU/L)[a]	53 ± 12	62 ± 32	>0.2[c]
Liver cirrhosis[b]	4 (36)	5 (38)	>0.2[d]
HBe antigen[b]	2 (18)	6 (46)	0.156[d]
Chronic hepatitis C	$n = 22$	$n = 50$	
Mean age (years)[a]	45 ± 9	46 ± 9	>0.2[c]
Sex (male:female)	12:10	17:33	0.101[c]
History of blood transfusion[b]	3 (14)	13 (26)	0.199[d]
Mean ALT level (IU/L)[a]	67 ± 31	46 ± 58	0.114[e]
Liver cirrhosis[b]	6 (27)	18 (36)	>0.2[e]
HCV RNA concentration (10^n copy/ml)[a]	3.0 ± 1.4	4.0 ± 0.9	<0.001[c]

*Concentration of HCV RNA was measured by competitive RT-PCR.
[a]Data are expressed as mean ± SD.
[b]Data are expressed as positive no. (%).
[c]Student's t-test.
[d]Fisher's exact test.
[e]Chi-square test.

TABLE III. Comparison of Clinical Background Between TTV DNA Positive and Negative Patients with Schistosomal Liver Disease

Background	TTV DNA positive	TTV DNA negative	*P*
Patients with HCV infection	$n = 9$	$n = 9$	
Mean age (years)[a]	44 ± 8	43 ± 6	>0.2[c]
Sex (male:female)	8:1	7:2	>0.2[d]
History of blood transfusion[b]	3 (33)	5 (56)	>0.2[d]
Mean ALT level (IU/L)[a]	60 ± 31	95 ± 64	0.159[c]
Liver cirrhosis[b]	3 (33)	5 (56)	>0.2[d]
Patients without HCV infection	$n = 5$	$n = 16$	
Mean age (years)[a]	47 ± 10	44 ± 13	>0.2[c]
Sex (male:female)	4:1	9:7	>0.2[d]
History of blood transfusion[b]	2 (40)	8 (50)	>0.2[d]
Mean ALT level (IU/L)[a]	28 ± 10	42 ± 22	0.189[c]
Liver cirrhosis[b]	2 (40)	5 (31)	>0.2[d]

[a]Data are expressed as mean ± SD.
[b]Data are expressed as positive no. (%).
[c]Student's t-test.
[d]Fisher's exact test.

TABLE IV. Comparison of Clinical and Virological Background Between TTV DNA Positive and Negative Volunteer Blood Donors*

Back ground	TTV DNA positive ($n = 32$)	TTV DNA negative ($n = 77$)	*P*
Mean age (years)[a]	30 ± 11	29 ± 7	>0.2[c]
Sex (male:female)	27:5	71:6	>0.2[d]
History of blood transfusion[b]	0	0	
Treatment history of schistosomiasis[b]	2 (6)	10 (13)	>0.2[e]
Mean ALT level (IU/L)[a]	26 ± 19	31 ± 48	>0.2[c]
Any HBV marker[b]	8 (25)	25 (33)	>0.2[d]
HCV RNA[b]	1 (3)	7 (9)	>0.2[e]
HCV antibody[b]	2 (6)	13 (17)	0.12[e]

*Any HBV marker includes HBs antigen, HBs antibody, and HBc antibody. But, 3 blood donors were positive for HBs antigen. All patients with HCV RNA were positive for HCV antibody.
[a]Data are expressed as mean ± SD.
[b]Data are expressed as positive no. (%).
[c]Student's t-test.
[d]Chi-square test.
[e]Fisher's exact test.

possibility of other route(s) such as the fecal-oral route [Okamoto et al., 1998a] that may explain the high prevalence of TTV DNA in volunteer blood donors who had no history of blood transfusion.

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